(2)

Institute Report No. 337

Acute Oral Toxicity of Triethyleneglycol Dinitrate (TEGDN) in ICR Mice

Earl W. Morgan, DVM, MAJ, VC
John R.G. Ryabik, BS, SP4
Conrad Wheeler, PhD
and
Don W. Korte, Jr., PhD, LTC, MSC

MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

May 1989

Toxicology Series: 132

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

This document has been approved for public release and sale; its distribution is unlimited.

Destroy this report when it is no longer needed. Do not return to the originator.

Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

This research was conducted in compliance with the "Guide for the Care and Use of Laboratory Animals." NIH Publication No. 85-23, as prepared by the Institute of Laboratory Animal Resources, National Research Council.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. *(AR 360-5)*

Edwin S. Beatrice 5 May 89

Edwin S. Beatrice (date)
COL, MC
Commanding

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT APPROVED FOR PUBLIC RELEASE; DISTRIBUTION IS UNLIMITED.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Institute Report No.: 337	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Mammalian Toxicology Division of Toxicology 6b. OFFICE SYMBOL (If applicable) SGRD-ULE-T	7a. NAME OF MONITORING ORGANIZATION US Army Biomedical Research and Development Laboratory
6c. ADDRESS (City, State, and ZIP Code) Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800	7b. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21701-5010
8a. NAME OF FUNDING/SPONSORING ORGANIZATION US Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 62720 / PROJECT NO. A835 / TASK NO. AB / WORK UNIT ACCESSION NO. DA303913
11. TITLE (Include Security Classification) (U) Acute Oral Toxicity of Triethyleneglycol Dinitrate (TEGDN) in ICR Mice	
12. PERSONAL AUTHOR(S) EW Morgan, JRG Ryabik, C Wheeler, and DW Korte, Jr.	
13a. TYPE OF REPORT Institute 13b. TIME COVERED FROM 23JAN85 TO 26APR85	14. DATE OF REPORT (Year, Month, Day) May 1989 15. PAGE COUNT 71
16. SUPPLEMENTARY NOTATION Toxicology Series No. 132	
17. COSATI CODES FIELD / GROUP / SUB-GROUP	18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number) Acute Oral Toxicity, TEGDN, Triethyleneglycol Dinitrate, Mouse, Mammalian Toxicology, Propellant

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The acute oral toxicity of triethyleneglycol dinitrate (TEGDN) was determined in male and female ICR mice by using the oral gavage single-dose method. The median lethal dose for male mice was 2036.5 ± 101.1 mg/kg and 1866.3 ± 86.2 mg/kg for female mice. TEGDN produced primarily reflexive and behavioral signs. These signs included increased startle reflex, depression of grasping and righting reflexes, inactivity, tremors, jumping , twitching, convulsions, and irritability. Other clinical signs associated with TEGDN administration were squinting, hunched posture, urine stains on the abdomen, and rough or dirty hair coat. The duration of clinical signs was acute. Most animals were exhibiting signs by 2 hours after dosing and had either died or had returned to normal by 24 hours after dosing. According to the classification scheme of Hodge and Sterner, these results place TEGDN in the slightly toxic class.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL EDWIN S. BEATRICE, COL, MC	22b. TELEPHONE (Include Area Code) (415) 561-3600 22c. OFFICE SYMBOL SGRD-ULZ

DD Form 1473, JUN 86 *Previous editions are obsolete.* SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

ABSTRACT

The acute oral toxicity of triethyleneglycol dinitrate (TEGDN) was determined in male and female ICR mice by using the oral gavage single-dose method. The median lethal dose for male mice was 2036.5 ± 101.1 mg/kg and 1866.3 ± 86.2 mg/kg for female mice. TEGDN produced primarily reflexive and behavioral signs. These signs included increased startle reflex, depression of grasping and righting reflexes, inactivity, tremors, jumping , twitching, convulsions, and irritability. Other clinical signs associated with TEGDN administration were squinting, hunched posture, urine stains on the abdomen, and rough or dirty hair coat. The duration of clinical signs was acute. Most animals were exhibiting signs by 2 hours after dosing and had either died or had returned to normal by 24 hours after dosing. According to the classification scheme of Hodge and Sterner, these results place TEGDN in the slightly toxic class.

Key Words: Acute Oral Toxicity, Triethyleneglycol Dinitrate, TEGDN, Mammalian Toxicology, Mouse

PREFACE

TYPE REPORT: Acute Oral Toxicity GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLB0

GLP STUDY NUMBER: 84010

STUDY DIRECTOR: LTC Don W. Korte Jr., PhD, MSC
Diplomate, American Board of Toxicology

PRINCIPAL INVESTIGATOR: CPT Earl W. Morgan, DVM, VC
Diplomate, American College of
Veterinary Preventive Medicine

CO-PRINCIPAL INVESTIGATOR: SP4 John R.G. Ryabik, BS

PATHOLOGIST: LTC Lance D. Lollini, DVM, MS, VC
Diplomate, American College of
Veterinary Pathologists

DATA MANAGER: Yvonne C. Johnson, BS

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, retired SOPs, raw data, analytical, stability, and purity data of the test compound, tissues, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Triethyleneglycol Dinitrate

INCLUSIVE STUDY DATES: 23 January - 26 April 1985

OBJECTIVE: The objective of this study was to determine the acute oral toxicity of triethyleneglycol dinitrate in male and female ICR mice.

ACKNOWLEDGMENTS

SSG James D. Justus, BS, SP4 James J. Fischer, and SP4 Scott L. Schwebe, provided research assistance and animal care; Conrad R. Wheeler, PhD and SP4 Paul B. Simboli, BS, provided chemical preparation and analysis; Richard A. Spieler and Charolette L. Speckman provided animal care and facility management; Colleen S. Kamiyama and Brenda V. Goce provided secretarial assistance. MAJ Larry D. Brown, VC, served as the LAIR Project Director for the acute toxicity studies on TEGDN.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS

We, the undersigned, declare that GLP Study 84010 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

20 DEC 85

DON W. KORTE JR, PhD / DATE
MAJ, MS
Study Director

20 Dec 85

JOHN R.G. RYABIK, BS / DATE
SP4, USA
Co-Principal Investigator

18 Dec 85

EARL W. MORGAN, DVM / DATE
CPT, VC
Principal Investigator

18 Dec 85

YVONNE C. JOHNSON, BS / DATE
DAC
Data Manager

LANCE O. LOLLINI, DVM / DATE
LTC, VC
Pathologist

19 Dec 85

CONRAD WHEELER, PhD / DATE
DAC
Analytical Chemist

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO ATTENTION OF

SGRD-ULZ-QA

5 May 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 84010

1. This is to certify that in relation ot LAIR GLP Study 84010, the following inspections were made:

24 February 1984	-	Protocol Review
31 January 1985	-	Weighing/Group Randomization
05 February 1985	-	Weighing/Dosing
23 April 1985	-	Weighing/Necropsy

2. The institute report titled "Triethylene Glycol Dinitrate in Mice," Toxicology Series 132, was audited on 13 January 1987.

Walter G. Bell

WALTER G. BELL
SFC, USA
Quality Assurance Auditor

TABLE OF CONTENTS

TABLE OF CONTENTS (cont.)

Acute Oral Toxicity of Triethyleneglycol Dinitrate in Male and Female in ICR Mice--Morgan *et al.*

INTRODUCTION

The Department of Defense is considering the use of diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in munition formulations. A "health effects" review conducted for the US Army Biomedical Research and Development Laboratory (USABRDL) identified numerous gaps in the toxicology database of these compounds (1). Consequently, USABRDL has tasked the Division of Toxicology, LAIR, to conduct an inital health effects evaluation of DEGDN, TMETN, TEGDN, and two DEGDN-based propellants, JA-2 and DIGL-RP. This initial evaluation includes the Ames mutagenicity assay, acute oral toxicity tests in rats and mice, a dermal toxicity test in rabbits, dermal and ocular irritation studies in rabbits, and dermal sensitization studies in guinea pigs.

Objective of Study

The objective of this study was to determine the acute oral toxicity of TEGDN in male and female ICR mice.

MATERIALS

Test Substance

Chemical name: Triethyleneglycol Dinitrate

Chemical Abstract Service Registry No.: 111-22-8

Chemical structure:

Molecular formula: $C_6H_{12}N_2O_8$

Source: Naval Ordnance Station
Indian Head, MD

Other test substance information is presented in Appendix A.

Vehicle

The vehicle for TEGDN was corn oil (Sigma Chemical Company, St. Louis, MO). The expiration date was April 1995.

Animal Data

Sixty-five male and 65 female ICR mice (Harlan Sprague-Dawley Inc, Indianapolis, IN), were used for this study. They were identified individually with ear tags numbered 85C00205 - 85C00324 inclusive, and 85C00074, 85C00097, 85C00103, 85C00114, 85C00133, 85C00158, 85C00166, 85C00169, 85C00173, and 85C00184. The vehicle control group was transferred from a previous study. The animal weights on receipt ranged from 19 to 33 g. Additional animal data appear in Appendix B.

Husbandry

Mice were caged individually in stainless steel wire mesh cages in racks equipped with automatically flushing dumptanks. The diet, fed *ad libitum*, consisted of Certified Purina Rodent Chow® Diet 5002 (Ralston Purina Company, Checkerboard Square, St Louis, MO); tap water was provided by continuous drip from a central line. The animal room temperature was maintained in a range from 22.2°C to 25.6°C with a relative humidity range of 38 to 51 percent. The photoperiod was 12 hours of light per day.

METHODS

Group Assignment/Acclimation

Study mice were randomized into 5 dose groups of 10 males and 10 females each and vehicle and cage control groups of 5 males and 5 females each. Allocation was accomplished by using a computer-based stratified weight-biased method. The Beckman TOXSYS® Animal Allocation Program was used in conjunction with a Beckman TOXSYS® Data Collection Terminal. The animals were acclimated for 13 days before the day of dosing. During this period they were observed daily for signs of illness.

Dose Levels

The results of the ALD determination suggested that the MLD was between 1900 and 2500 mg per kg. Based on these data, test doses were selected (Table 1).

TABLE 1: TEGDN Doses

Group	Dosage Level mg/kg
1	2150
2	2450
3	2780
4	3160
5	1670
6	1290
7 (cage control)	
8 (vehicle control from earlier study)	

Compound Preparation

TEGDN was received as a 10% solution in ethanol. The ethanol was removed with a rotary evaporator. TEGDN was then suspended in corn oil using a vortex to form a viscous clear yellow-to-light-brown oil emulsion. The compound readily went into suspension and there was no discernible separation throughout the dosing procedures.

Chemical Analysis of Dosing Solution

NMR analysis demonstrated that the neat compound is stable for at least 1 month (Appendix A). Test for homogeneity of the test compound in the vehicle was conducted. The deviation of individual values from the mean of each set of 3 samples (top, middle, bottom) did not exceed 3.8% for any emulsion.

Test Procedures

This study was conducted in accordance with EPA guidelines (2) and LAIR SOP-OP-STX-36 (3).

The volume of dosing solution each animal received was based upon the desired dose level, the compound concentration in suspension, and animal weights. The dose level was increased by varying the concentration of each suspension. Volumes ranged from 0.26 to 0.36 ml in the males and 0.23 to 0.31 ml in females. The vehicle control group was given 0.24 to 0.33 ml of corn oil. The volumes given were based on a dose of 10 ml per kg. The cage control group was untreated. Dosing was performed by the oral gavage method without animal sedation or anesthesia. Sterile disposable syringes (Becton, Dickenson & Co., Rutherford, NJ) fitted with 20-gauge, 1-1/2 inch, ball-tipped feeding tubes (Popper & Sons, Inc, New Hyde Park, NY) were utilized. Animals in groups 1-4 were dosed between 0900 and 1031 hours on 9 April 1985. Group 5 mice (1670 mg/kg) were dosed between 1006 and 1024 on 10 April 1985 and group 6 (1290 mg/kg) mice were dosed between 1009 and 1017 on 12 April 1985 after analysis of the data from groups 1-4.

Observations

Observations for mortality and signs of acute toxicity were performed daily according to the following procedure: (a) animals were observed undisturbed in their cages, (b) animals were removed from their cages and given a physical examination, and (c) animals were observed after being returned to their cages. On the day of dosing, the animals were checked intermittently throughout the day. Recorded observations were performed 1, 2, and 4 hours after dosing and daily for the remainder of the 2-week test period. A second "walk through" observation was performed daily with only significant observations recorded. Body weights were recorded weekly during the course of the study.

Necropsy

Animals that died during the observation period were submitted for a complete gross necropsy. Those which survived the 14-day study period were submitted for necropsy immediately after sacrifice by barbituate overdose.

Statistical Analysis

Statistical analyses were performed on the study results. The calculated lethal doses were derived by probit analysis using the maximum likelihood method, as described by Finney (4). The program, PROBIT, developed for the Data General Computer, Model MV8000, was used to plot the probit curve and lethal dose values.

Duration of Study

Appendix C is a complete listing of historical events.

Changes/Deviations

The dosing phase of this study was accomplished according to the protocol and applicable amendments with the following exceptions: The animals that were originally planned for the vehicle control group were dosed with 1290 mg/kg of TEGDN in order to define the lower end of the dose range more accurately. The cage control group was reduced from 10 to 7 animals due to the loss of 2 mice during quarantine and the need to replace one animal in Group 1 due to misdosing. The vehicle control group from a previous set of animals was used as a historical control.

Storage of Raw Data and Final Report

A copy of the final report, study protocols, raw data, retired SOPs and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

Mortality

Seventy-five animals died as a result of the dosing. Sixty-two (82.7%) deaths occurred within 12 hours of dosing. An additional 11 (14.6%) deaths occurred by 25 hours after dosing and the last 2 (2.7%) deaths occurred by 48 hours after dosing. Table 2 lists the compound-related deaths by group and the percent mortality. Appendix D is a tabular presentation of cumulative mortality.

TABLE 2: Compound Related Deaths by Group

GROUP	Dose Level mg/kg	Compound Related Death/ Number in Group	Percent Mortality
		MALE	
6	1290	0/5	0
5	1670	2/10	20
1	2150	7/10	70
2	2450	6/10	60
3	2780	10/10	100
4	3160	10/10	100
7	Cage Control	0/4	0
	Vehicle Control	0/5	0
		FEMALE	
6	1290	0/5	0
5	1670	3/10	30
1	2150	7/10	70
2	2450	10/10	100
3	2780	10/10	100
4	3160	10/10	100
7	Cage Control	0/3	0
	Vehicle Control	0/4	0

Lethal Dose Calculations

Lethal dose values were calculated by probit analysis and the equation for the probit regression line was: $Y = -31.56 + 11.05 \log X$ for males and $Y = -42.33 + 14.47 \log X$ for females, where X is the dose and Y the corresponding probit value. Misdosed animals were excluded from statistical analysis and eliminated from the study. Lethal doses calculated from the equation for the probit regression line are presented in Table 3. Figures 1 and 2 graphically present the actual data points and the regression line.

TABLE 3: Calculated Lethal Doses (LD) of TEGDN in ICR Mice

Level	Calculated Dose* (mg/kg)	95% Confidence Limits (mg(base)/kg)
MALES		
LD10	1559.1 ± 141.3	(1153.3, 1777.1)
LD50	2036.5 ± 101.1	(1791.0, 2233.2)
LD90	2660.0 ± 172.1	(2404.4, 3246.1)
FEMALES		
LD10	1521.9 ± 118.5	(1160.9, 1702.6)
LD50	1866.3 ± 86.2	(1651.9, 2037.3)
LD90	2288.5 ± 130.8	(2090.1, 2741.8)

* Calculated dose ± standard error.

Figure 1

TEGDN Dose Response Curve in Male ICR Mice

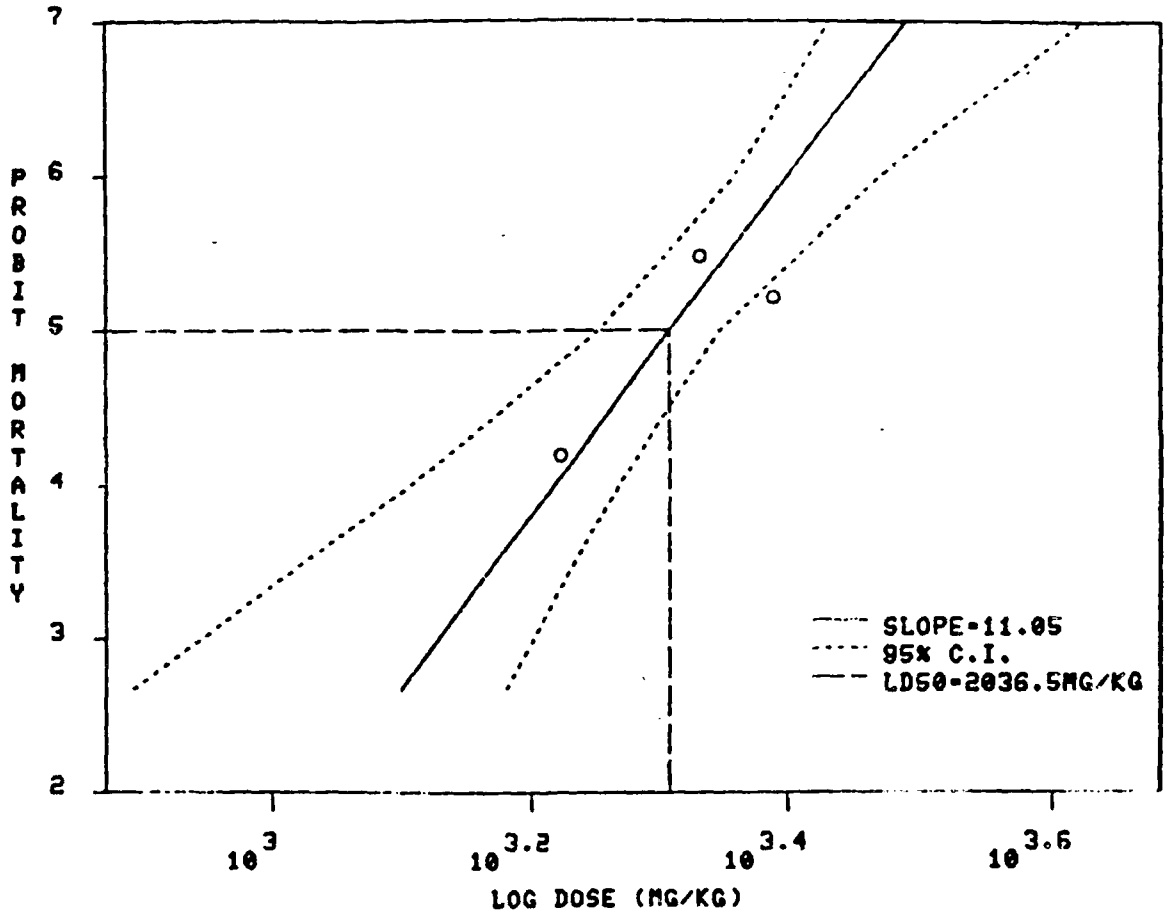
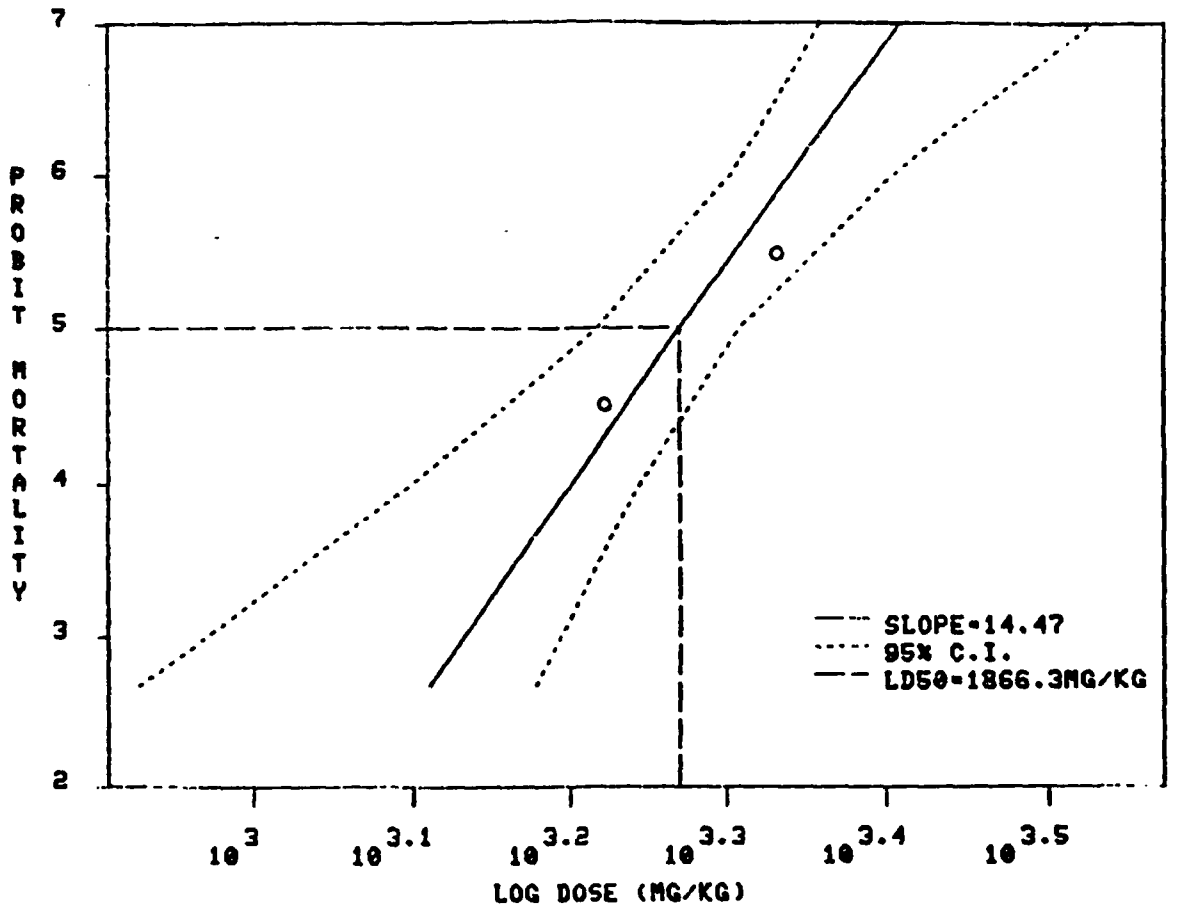


Figure 2

TEGDN Dose Response Curve in Female ICR Mice



Clinical Observations

The most frequently observed categories of clinical signs were reflexes (63 of 110 animals dosed) and behavioral (77 of 110). Reflexive signs included depressed grasping and righting reflexes, and increased startle reflex. Behavioral signs included tremors, inactivity, jumping, twitching, and irritability. All animals that died exhibited one or more reflexive and/or behavioral signs or had progressed to a prostrate/moribund condition or death before the first observation for clinical signs. Although there appeared to be a dose response relationship for these signs, the rapid onset of death in the higher dose groups precluded statistical confirmation of this inference. However, the presence of convulsions in the higher dose groups supports a dose-response relationship for these clinical signs.

Most other clinical signs were attributable to a more generalized response of malaise and discomfort associated with TEGDN administration. These signs included squinting (63 of 110), hunched posture (51 of 110), urine on the abdomen (27 of 110), and rough or dirty hair coat (10 of 110). Squinting exhibited an apparent dose response relationship. The higher frequency that urine was observed on the abdomen of the male mice is primarily a function of anatomical differences between the sexes.

Thirteen animals (12 females and 1 male) died before any clinical signs were recorded. One female was in the 1670 mg/kg group; all others were in the 2450 mg/kg or higher groups.

With the exception of the animals that ultimately died most clinical signs had cleared by 24 hours after dosing. One animal in the 1670 mg/kg group remained hyperactive through Day 4 after dosing. On day 6 after dosing, one male in the 2450 mg/kg group developed a rough and dirty hair coat which persisted to the end of the study. Due to a water lixit malfunction, one male in the 1670 mg/kg group developed a rough coat on Day 12 after dosing. His condition progressed to tremors, hunched posture, and marked dehydration by the following day before the malfunction problem was found and corrected. Tables 4 and 5 contain a summary of clinical observations. Appendix E contains individual animal histories.

Weight gains of survivors were not significantly affected by dosing. Table 6 presents the mean body weights by groups. Appendix F contains individual weight tables.

TABLE 4: Incidence Summary for Clinical Observations in Male Mice Administered TEGDN

Clinical Signs	Group	6	5	1	2	3	4	7 (Controls)	
	Dose (mg/kg)	1290	1670	2150	2450	2780	3160	Cage	Vehicle
	(n=)	5	10	10	10	10	10	4	5
Reflexes[1]		1	7	7	8	9	7		
Behavioral[2]		1	8	10	10	10	8		
Squinting		2	5	6	7	10	9		
Urine Abdomen		2	6	2	3	6	3		
Hunched Posture		1	5	6	7	7	6		
Rough Coat		1	3	2	2	1			1
Prostrate		1	1	3	1	4	4		
Tonic/Clonic Convulsions					1	2			
Other[3]		1	1	1			1		
Died Without Any Signs							1		
Normal Throughout		2						4	4

[1] Includes depressed grasping, depressed righting, and increased startle reflexes.
[2] Includes irritability, inactivity, tremors, twitching, jumping, and hyperactivity.
[3] Includes skin ulceration/scabbing, dehydration, gasping, and increased salivation.

TABLE 5: Incidence Summary for Clinical Observations in Female Mice Administered TEGDN

Clinical Signs	Group	6	5	1	2	3	4	7 (Controls)	
	Dose (mg/kg)	1290	1670	2150	2450	2780	3160	Cage	Vehicle
	(n=)	5	10	10	10	10	10	3	4
Reflexes[1]		2	3	7	6	4	2		
Behavioral[2]		3	3	8	7	7	2		1
Squinting		3	3	7	5	4	2		
Urine Abdomen			2	1	1	2			
Hunched Posture		3	2	6	5	2	1		
Prostrate/Moribund				2	3	4	3		
Other[3]				1	1	1			
Died Without Any Signs				1	3	2	6		
Normal Throughout		2	6					3	3

[1] Includes depressed grasping, depressed righting, and increased startle reflexes.
[2] Includes irritability, inactivity, tremors, twitching, and jumping.
[3] Includes rough coat, tonic convulsions, and alopecia on head.

TABLE 6: Mean Body Weights in Grams ± S.E (N)

MALES

Dose Groups (mg/kg)	At Receipt	Dosing Day 0	Midtrial Day 7	Termination Day 14*
1290	27.0 ±0.4(5)	32.4 ±0.5(5)	33.6 ±0.7(5)†	35.0 ±0.3(5)
1670	27.0 ±0.7(10)	29.6 ±0.8(10)	31.9 ±1.0(8)	32.3 ±1.8(8)
2150	27.8 ±0.9(10)	31.3 ±0.9(10)	37.3 ±2.9(3)	37.0 ±2.1(3)
2450	27.6 ±0.5(10)	30.0 ±0.9(10)	33.3 ±1.6(4)	35.0 ±1.5(4)
2780	28.2 ±0.6(10)	30.8 ±0.7(10)	--	--
3160	27.1 ±0.5(10)	28.6 ±0.7(10)	--	--
Vehicle Control	25.2 ±0.4(5)	32.0 ±0.3(5)	34.6 ±0.4(5)	35.0 ±0.5(5)
Cage Control	27.5 ±0.9(5)	33.3 ±0.8(5)	34.3 ±0.9(5)	34.5 ±1.1(5)

* Weights after overnight fast.
† Day 4

TABLE 7: Mean Body Weights in Grams ± S.E (N)

FEMALES

Dose Groups (mg/kg)	At Receipt	Dosing Day 0	Midtrial Day 7	Termination Day 14*
1290	26.0 ±0.8(5)	27.8 ±0.6(5)	29.8 ±0.9(5)†	30.2 ±0.4(5)
1670	26.3 ±0.6(10)	27.4 ±0.7(10)	29.1 ±0.8(7)	30.4 ±1.0(7)
2150	27.0 ±0.9(10)	28.7 ±0.7(10)	31.3 ±0.7(3)	31.7 ±0.7(3)
2450	25.9 ±0.7(10)	27.4 ±0.8(10)	--	--
2780	25.0 ±1.0(10)	26.7 ±0.8(10)	--	--
3160	26.1 ±0.6(10)	26.8 ±0.7(10)	--	--
Vehicle Control	22.4 ±0.7(5)	25.0 ±0.5(5)	26.5 ±0.3(4)	28.5 ±0.5(4)
Cage Control	26.7 ±1.1(3)	30.0 ±0.6(3)	30.3 ±0.3(3)	29.7 ±0.7(3)

* Weights after overnight fast.
† Day 4

Gross Pathological Observations

The mortalities which occurred after dosing appear to have been caused by the test compound. No remarkable gross lesions were found in any of the animals. The veterinary pathologist's report appears in Appendix G.

DISCUSSION

The calculated median lethal dose for TEGDN was 2036.5 mg/kg in male ICR mice and 1866.3 mg/kg in female ICR mice. These values place TEGDN within the slightly toxic classification (5).

Clinical signs indicated that TEGDN primarily affected the central nervous system. Clinical signs included inactivity, tremors, increased startle reflex, jumping, twitching, depression of grasping and righting reflexes, irritability, and convulsions. These observations correspond closely to the results reported by Andersen *et al.* (6). They reported that rats given TEGDN intraperitoneally displayed hyperactivity to both auditory and tactile stimuli accompanied by tremors and severe clonic convulsions. They also reported that the results of pentobarbital sleep time studies indicated a depressant activity by TEGDN on the central nervous system.

The mice also exhibited a syndrome of general malaise/ discomfort as indicated by the observations of squinting, hunched posture, rough coat, and urine stains on the abdomen. These signs were more prevalent in the 2150 mg/kg and higher dose groups.

CONCLUSION

Triethyleneglycol dinitrate is a slightly toxic compound that produces primarily central nervous system symptoms. Calculated MLD values were 2036.5 mg/kg in male ICR mice and 1866.3 mg/kg in female ICR mice.

REFERENCES

1. Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No. ADA 127846.

2. Environmental Protection Agency. Office of Pesticides and Toxic Substances, Office of Toxic Substances (TS-792). Acute exposure, oral toxicity. In: Health effects test guidelines. Washington, DC: Environmental Protection Agency, August 1982; EPA 560/6-82-001.

3. Acute oral toxicity study (ALD and LD50). LAIR Standard Operating Procedure OP-STX-36, Letterman Army Institute of Research, Presidio of San Francisco, CA. 15 June 1984.

4. Finney DJ. Probit analysis. 3rd ed. Cambridge: Cambridge University Press, 1971:20-80.

5. Hodge HC, Sterner JH. Tabulation of toxicity classes. Am Ind Hyg Ass Q 1943; 10:93-96.

6. Andersen M, Koppenhaver, RE, Jenkins LJ, Jr. Some neurotoxic properties of triethylene glycol dinitrate: a comparison with decamethonium. Toxicol Appl Pharmacol 1976; 36:585-594.

Appendix A: CHEMICAL DATA

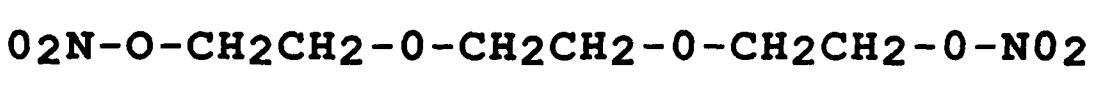
Chemical Name: Ethanol, 2,2'-[1,2-ethanediylbis(oxy)] bis-, dinitrate

Alternate Chemical Names: Triethyleneglycol dinitrate, NOSET-A

Chemical Abstracts Service Registry No.: 111-22-8

LAIR Code Number: TA44

Chemical Structure:



Molecular Formula: $C_6H_{12}N_2O_8$

Molecular Weight: 240

Physical State: Yellow oil

Density: (g/cm^3): 1.32*

Manufacturer: Naval Ordnance Station
Indian Head, MD

Lot No.: 130-84

---

* Holleman JW, Ross RH, Carroll JW. Problems definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No. ADA 127846, p17.

Appendix A (cont.): CHEMICAL DATA

Analytical data: The compound chromatographed as a single peak (retention time 5.8 min) by HPLC analysis under the following conditions: column, Brownlee RP-18 (4.6 x 250 mm); solvent system, 30% water, 70% methanol; flow rate 0.9 ml/min, detection wavelength, 215 nm.[†] No impurities were detectable by NMR.[‡] NMR (80 MHz, $CDCl_3$): 3.65 (S, 4H, $-CH_2-O-CH_2CH_2-O-CH_2-$), 3.72-3.84 (Complex multiplet, 4H, terminal methylene groups). IR (KBr): 2900, 1630, 1280, 1130, 1030, 910, 860 cm.[§]

Stability: The compound was received as a 10% solution in ethanol. Periodic analysis of this solution by HPLC has shown no evidence of decomposition to date (4 months).[†] NMR analysis demonstrated that the neat compound is stable for at least 1 month.[‡]

[†] Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.1, p26-30, 42-43. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[‡] Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p63. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[§] Ibid. p64.

Appendix A (cont.): CHEMICAL DATA

Analysis of TEGDN Dosing Formulations

INTRODUCTION:

Emulsions of triethylene glycol dinitrate (TEGDN) in corn oil were prepared by shaking or stirring mixtures of the two components. The emulsions were subsequently used for dosing animals in the GLP Studies #84010 (acute oral toxicity in mice) and #84011 (acute oral toxicity in rats). After dosing, the remainder of each emulsion was stored at 4°C for analysis. Determination of the TEGDN concentration was accomplished by reverse-phase liquid chromatography.

MATERIALS:

Chromatographic analysis was performed using a Hewlett-Packard 1090 high pressure liquid chromatography (HPLC) system with diode array detector (Hewlett-Packard, Palo Alto, CA). Separations were obtained on a Brownlee RP-18 column (4.6 x 250 mm, Brownlee Labs, Inc., Santa Clara, CA). HPLC grade acetonitrile and water were obtained from the J.T. Baker Chemical Co., Phillipsburg, NJ.

METHODS:

Analysis of TEGDN solutions was accomplished under the following HPLC conditions: solvent, 70% acetonitrile-30% water; solvent flow, 0.9 ml/min; injection volume, 10 µL; detector wavelength, 205 nm. The HPLC mobile phase was used to prepare standards as well as to extract the TMGDN/corn oil mixtures. Standards were prepared by weighing TEGDN on aluminium foil (0.5 mm squares) using a microbalance. The weigh boats containing TEGDN were added to volumetric flasks. The flasks were filled to volume and the contents mixed well by shaking. The concentration of the standards covering this ranged from 10.4 to 561.2 µg/ml and a set of standards covering this range was analyzed both before and after each set of samples (diluted dosing emulsions).

To extract the dosing preparations the TEDGN/corn oil mixtures were removed from the refrigerator and warmed to room temperature. After rapidly stirring each sample for a minimum of five minutes an aliquot of approximately one ml was removed and transfered to a tared volumetric flask. The weight of each aliquot transferred was recorded and the flask filled to volume. A second dilution was required prior to analysis by HPLC.

Appendix A (cont.): CHEMICAL DATA

Analysis of TEGDN Dosing Formulations (cont.)

To determine if the emulsions of TEGDN in corn oil prepared for dosing were homogenous, a series of emulsions was prepared with TEGDN concentrations spanning the range of concentrations employed in the dosing preparations. Four emulsions containing 50, 200, 400 and 800 mg of TEGDN per ml were prepared in 20 ml scintillation vials. After stirring with a magnetic stir bar for at least 5 min, aliquots from the top, middle, and bottom of the emulsions were removed and transfered to tared 25 ml volumetric flasks. The exact weight of the aliquot was recorded and the flask filled to volume. One ml of this solution was transfered to a second volumetric flask for further dilution prior to HPLC analysis.

RESULTS

Under the conditions of the analysis TEGDN eluted with a retention time of 4.4 min. A plot of the TEGDN concentration versus peak area was linear within the range of concentrations (10.4.to 561.2 µg/ml) employed as standards. Consecutive analyses (n = 10) were performed with standards containing 103.24, 301.84 and 608.2 µg TEGDN/ml. The coefficient of variation for each set of peak area values was 0.58%, 0.38%, and 0.27%, respectively. Standards were analyzed both before and after the analysis of samples prepared from dosing emulsions. The differences in peak areas between corresponding standards run before and after was less than 1%. As can be seen at the bottom of Tables 1 and 2 the standard plot was virtually identical from assay to assay.

Extraction of the dosing emulsions with 70% acetonitrile-30% water resulted in quantitative recovery of TEGDN with no peaks in the chromatogram from corn oil. The results for the determination of homogeneity are presented in Table 1. The deviation of individual values from the mean of each set of three samples (top, middle, bottom) did not exceed 3.8% for any emulsion prepared.

DISCUSSION

The quantitative recovery of TEGDN from corn oil emulsions, in addition to the linearity and reproducibility of the calibration plot over the period of the study, indicates the assay is a valid method to quantitate TEGDN. The data in Table 1 demonstrates that the dispersion of TEGDN in corn oil

Appendix A (cont.): CHEMICAL DATA

Analysis of TEGDN Dosing Formulations (cont.)

provides a homogenous emulsion over a range of 50 to 800 mg/ml. Since the dosing preparations were prepared in an identical manner, they were, by implication, homogenous.

The data from the analysis of the dosing emulsions is presented in Table 2. For several samples the concentration of TEGDN determined by analysis showed significant deviation from the target concentration. Of the thirteen emulsions analyzed three showed deviations of greater than 10% from the target concentration. Reanalysis of two of these samples yielded results within three percent of the original values, thus confirming the initial results.

REFERENCE

Wheeler CR. Nitrocellulose - Nitroguanidine Projects. Laboratory Notebook #85-01-006, p 1-27 Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

Analysis of TEGDN Dosing Formulations (cont.)

CALCULATIONS

A series of standards were analyzed before and after the samples (diluted dosing emulsions) for each study. The two peak area values for each standard solution were averaged and linear least-squares regression performed on the concentration versus peak area data. This provided the equation for the best fitting line in the form of Equation 1 in which:

Equation 1 $$Y \text{ (peak area)} = mX + b$$

m is the slope, X is the concentration (µg/ml) and b is the intercept. The concentration of TEGDN in the final dilution was calculated by substituting for Y the peak area obtained from HPLC analysis and solving for X.

The total amount of TEGDN in the sample analyzed was then calculated as shown in Equation 2.

Equation 2 $$\text{Total TEGDN (mg)} = \frac{X \times (\text{dilution factor})}{10^3\ \mu g/mg} = Y$$

The volume corresponding to the weight of TEGDN calculated above was determined by dividing by the density of TEGDN (Equation 3).

Equation 3 $$\text{Total TEGDN (ml)} = \frac{\text{Total TEGDN (mg)}}{1320\ mg/ml} = \frac{Y}{1320} = Z$$

The contribution by corn oil to the volume of the original aliquot of emulsion removed for analysis was calculated as follows (Equation 4)

$$\text{Volume of corn oil} = \frac{\text{Weight of aliquot removed for analysis} - \text{Weight of TEGDN}}{\text{Density of corn oil}} = \frac{\text{Weight of aliquot} - Y}{0.918\ g/ml} = V$$

The concentration of TEGDN could then be determined as follows:

Equation 5

$$\text{Conc. of TEGDN (mg/ml)} = \frac{\text{mg TEGDN}}{\text{total volume of aliquot removed for analysis}} = \frac{\text{mg TEGDN}}{\text{ml TEGDN + corn oil}} = \frac{Y}{Z + V}$$

Appendix A (cont.): CHEMICAL DATA

Analysis of TEGDN Dosing Formulations (cont.)

Table 1

Assessment of homogeneity for TEGDN/corn oil emulsions. Aliquots of approximately 1 ml were withdrawn from the top (T), middle (M), and bottom (B) of the emulsions prepared to represent the range of TEGDN concentrations [TEGDN] employed in dosing. Three samples were analyzed from the top layer to determine the within-layer variability in the sampling and analysis.

Target [TEGDN] (mg/ml)	Site of sampling	[TEGDN] (mg/ml) determined by analysis	Mean [TEGDN] (mg/ml) of top samples $(T+T+T)/3=X_T$	Mean [TEGDN] (mg/ml) of all samples $(X_T + M + B)/3$	Deviation from mean (%)
	T	45.8			
	T	47.4	47.0	47.3	-0.6%
50*	T	47.7			
	M	48.2			+1.9%
	B	46.8			-1.1%
	T	205.2			
	T	209.6	207.9	210.4	-1.2%
200†	T	209.0			
	M	211.4			+1.5%
	B	212.0			+0.8%
	T	426.0			
	T	418.8	421.0	423.4	-0.6%
400‡	T	418.3			
	M	419.4			-0.9%
	B	429.7			+1.5%
	T	805.7			
	T	824.7	801.6	833.3	-3.8%
800‡	T	774.3			
	M	844.6			+1.4%
	B	853.6			+2.4%

*Equation for the standard curve: $Y = 0.0453X + 0.2426$.
†Equation for the standard curve: $Y = 0.0465X - 0.0622$.
‡Equation for the standard curve: $Y = 0.0459X + 0.0639$.

Appendix A (cont.): CHEMICAL DATA

Analysis of TEGDN Dosing Formulations (cont.)

TABLE 2

Concentration of TEGDN [TEGDN] in dosing emulsions prepared for GLP Studies 84010 and 84011. Samples that were analyzed a second time for verification have been denoted with a R (reanalyzed) in front of the target concentration. In each case reanalysis yielded a value for concentration that was within 3% of the initial determination.

Study Number	Target [TEGDN] (mg/ml)	Date Prepared	Date Analyzed*	[TEGDN] determined by Analysis (mg/ml)	% of Target [TEGDN]
	215.0	8 Apr 85	10 Jun 85	237.8	110.6
	245.0	8 Apr 85	10 Jun 85	257.3	105.0
	278.0	8 Apr 85	10 Jun 85	283.2	101.9
84010	316.0	8 Apr 85	10 Jun 85	334.1	105.7
	167.0	9 Apr 85	1 Jul 85	169.4	101.4
	360.0	9 Apr 85	10 Jun 85	392.6	109.1
	129.0	12 Apr 85	1 Jul 85	133.3	103.3

Study Number	Target [TEGDN] (mg/ml)	Date Prepared	Date Analyzed*	[TEGDN] Determined by Analysis (mg/ml)	% of Target [TEGDN]
	212.0	26 Mar 85	14 Aug 85	227.5	107.3
	234.0	26 Mar 85	14 Aug 85	210.6	90.0
	R 257.0	26 Mar 85	14 Aug 85	329.1	128.1
84011	285.0	26 Mar 85	14 Aug 85	284.1	99.7
	R 378.0	26 Mar 85	14 Aug 85	417.2	110.4
	428.0	4 Apr 85	14 Aug 85	461.6	107.9

*The correlation coefficient and equation of the line for the standard plot on each date of analysis is given as follows:

Date of Analysis	Correlation Coefficient	Equation
10 Jun 85	0.9999	y = 0.0454 X + 0.0877
1 Jul 85	0.9999	y = 0.0455 X + 0.0527
28 Jul 85	0.9999	y = 0.0453 X + 0.0783
17 Jul 85	0.9999	y = 0.0454 X + 0.0385
25 Jun 85	0.9999	y = 0.0454 X + 0.0746
14 Aug 85	0.9999	y = 0.0461 X + 0.0838

Appendix B: ANIMAL DATA

Species: *Mus musculus*

Strain: ICR

Source: Harlan Sprague-Dawley, Inc
Indianapolis, IN

Sex: Male and female.

Dates of birth: Males: 8 February 1985
Females: 1 February 1985
Vehicle Controls: 7 December 1984

Method of randomization: Weight bias, stratified animal allocation (RANDOM Computer Program, SOP OP-ISG-21)

Animals in each group: 10 male and 10 female animals - Groups 1-5; 5 male and 5 female animals - Group 6 and vehicle control group; 4 males and 3 females - cage control group

Condition of animals at start of study: Normal

Body weight range at dosing: 23 - 36 g

Identification procedure: Cervical tag

Pretest conditioning: Quarantine/acclimation 27 March - 9 April 1985

Justification: The laboratory mouse has proven to be a sensitive and reliable system for lethal dose determinations.

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

Date	Event
27 Mar 85	ICR mice for GLP Protocol 84035 were received. Mice were checked for physical condition, sexed, weighed, tagged, and individually caged.
28 Mar 85	Four mice (2 male and 2 female) were submitted for necropsy quality control.
28 Mar - 8 Apr 85	Animals were observed daily.
4 Apr 85	Sixty males and 58 females were transferred to GLP Protocol 84010. Animals were weighed and randomized into dose groups.
9 Apr 85	Group 1-4 animals were fasted 4 hours, weighed, dosed, and observed at 1, 2, and 4 hours after dosing.
10 Apr 85	Group 5 animals were fasted 4 hours, weighed, dosed, and observed at 1, 2, and 4 hours after dosing.
12 Apr 85	Group 6 animals were fasted 4 hours, weighed, dosed, and observed at 1, 2, and 4 hours after dosing.
10-26 Apr 85	All animals were observed daily in a.m. and p.m.
16 Apr 85	All animals were weighed.
23 Apr 85	All surviving animals in Groups 1-4 and 7 were weighed, sacrificed, and submitted for necropsy.
24 Apr 85	All surviving animals in Group 5 were weighed, sacrificed, and submitted for necropsy.
26 Apr 85	All surviving animals in Group 6 were weighed, sacrificed, and submitted for necropsy.

Appendix D: CUMULATIVE MORTALITY DATA (deaths/group)

Dose mg/kg	Animals/ Group	Time After Dosing								
		Hours				Days				
		1	2	4	12	1	2	3	4	5-14
MALES										
1290	5	0	0	0	0	0	0	0	0	0
1670	10	0	0	0	0	2	2	2	2	2
2150	10	0	1	2	4	6	7	7	7	7
2450	10	0	0	3	4	6	6	6	6	6
2780	10	0	0	5	10	10	10	10	10	10
3160	10	1	4	5	9	10	10	10	10	10
Vehicle	5	0	0	0	0	0	0	0	0	0
Cage	5	0	0	0	0	0	0	0	0	0
FEMALES										
1290	5	0	0	0	0	0	0	0	0	0
1670	10	0	0	0	0	2	3	3	3	3
2150	10	0	1	5	7	7	7	7	7	7
2450	10	0	3	6	9	10	10	10	10	10
2780	10	0	2	7	7	10	10	10	10	10
3160	10	0	5	8	9	10	10	10	10	10
Vehicle	5	0	0	0	0	0	0	0	0	0
Cage	3	0	0	0	0	0	0	0	0	0
TOTAL		1	16	41	62	73	75	75	75	75

Appendix E: INDIVIDUAL ANIMAL HISTORIES

MALE CONTROL TRIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00243†	NORMAL	8-23 APRIL	
85C00244†	NORMAL	8-23 APRIL	
85C00258†	NORMAL	8-23 APRIL	
85C00259†	NORMAL	8-23 APRIL	
85C00074	ROUGH COAT	5,6 FEBRUARY	SLIGHT
85C00097	NORMAL	4-19 FEBRUARY	
85C00103	NORMAL	4-19 FEBRUARY	
85C00114	NORMAL	4-19 FEBRUARY	
85C00133	NORMAL	4-19 FEBRUARY	

† Cage controls, remaining animals are vehicle controls.

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE 1290 MG/KG TRIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00236	NORMAL	8-26 APRIL	
85C00240	URINE ABDOMEN	12 APRIL	SLIGHT
	SQUINTING	12 APRIL	SLIGHT
85C00246	ROUGH COAT	12 APRIL	SLIGHT
	ULCERATION, BACK	13,14 APRIL	SLIGHT
	SCAB,BACK	15-24 APRIL	SLIGHT
85C00247	INACTIVE	12 APRIL	MODERATE
	SQUINTING	12 APRIL	MARKED
	TREMORS	12 APRIL	MODERATE
	HUNCHED POSTURE	12 APRIL	MARKED
	DEP. GRASPING REFLEX	12 APRIL	MARKED
	PROSTRATE	12 APRIL	
	URINE ABDOMEN	12 APRIL	SLIGHT
85C00249	NORMAL	8-26 April	

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE 1670 MG/KG TRIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00218	DEP. GRASPING REFLEX	10 APRIL	SLIGHT
	SQUINTING	10 APRIL	SLIGHT
	INACTIVE	10 APRIL	SLIGHT
85C00227	HYPERACTIVE	10 APRIL	MODERATE
	HUNCHED POSTURE	10 APRIL	SLIGHT
	URINE ABDOMEN	10 APRIL	SLIGHT
	HYPERACTIVE	11-14 APRIL	SLIGHT
85C00228	INACTIVE	10 APRIL	MODERATE
	HUNCHED POSTURE	10 APRIL	MODERATE
	DEP. GRASPING REFLEX	10 APRIL	MODERATE
	SQUINTING	10 APRIL	MODERATE
	URINE ABDOMEN	10,11 APRIL	SLIGHT
	TREMORS	10 APRIL	MODERATE
	TWITCHING	10 APRIL	SLIGHT
85C00235	INACTIVE	10 APRIL	MODERATE
	HUNCHED POSTURED	10 APRIL	MODERATE
	TREMORS	10 APRIL	MODERATE
	DEP. GRASPING REFLEX	10 APRIL	MARKED
	SQUINTING	10 APRIL	MODERATE
	DEATH	11 APRIL	
85C00239	DEP. GRASPING REFLEX	10 APRIL	SLIGHT
	URINE ABDOMEN	10 APRIL	MODERATE
	INACTIVE	10 APRIL	MODERATE
85C00245	INACTIVE	10 APRIL	MARKED
	TREMORS	10 APRIL	SLIGHT
	HUNCHED POSTURE	10 APRIL	SLIGHT
	SQUINTING	10,11 APRIL	MODERATE
	URINE ABDOMEN	10,11 APRIL	MARKED
	DEP. GRASPING REFLEX	10,11 APRIL	MODERATE
	PROSTRATE	11 APRIL	
	DEATH	11 APRIL	

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE 1670 MG/KG TRIETHYLENEGLYCOL DINITRATE (CONT.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00255	HYPERACTIVE	10 APRIL	SLIGHT
	TREMORS	10 APRIL	MODERATE
	SQUINTING	10 APRIL	SLIGHT
	DEP. GRASPING REFLEX	10 APRIL	MODERATE
	INACTIVE	10 APRIL	SLIGHT
	INC. STARTLE REFLEX	10 APRIL	SLIGHT
85C00256	ROUGH COAT	10 APRIL	SLIGHT
	URINE ABDOMEN	10 APRIL	SLIGHT
85C00263	ROUGH COAT	10 APRIL	SLIGHT
85C00264	INACTIVE	10 APRIL	SLIGHT
	HUNCHED POSTURE	10 APRIL	SLIGHT
	DEP. GRASPING REFLEX	10 APRIL	SLIGHT
	URINE ABDOMEN	10 APRIL	SLIGHT
	ROUGH COAT	22-24 APRIL	MARKED
	TREMORS	23 APRIL	MODERATE
	HUNCHED POSTURE	23,24 APRIL	MODERATE
	DEHYDRATED	23 APRIL	

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE 2150 MG/KG TRIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00217	PROSTRATE	9 APRIL	
	TREMORS	9 APRIL	MARKED
	GASPING	9 APRIL	MARKED
	DEATH	9 APRIL	2.4 HOURS
85C00223	HUNCHED POSTURE	9 APRIL	SLIGHT
	SQUINTING	9 APRIL	MODERATE
	URINE ABDOMEN	9 APRIL	MODERATE
	INACTIVE	9 APRIL	MODERATE
85C00226	HUNCHED POSTURE	9 APRIL	SLIGHT
	TREMORS	9 APRIL	MARKED
	INACTIVE	9 APRIL	MODERATE
	DEP. GRASPING REFLEX	9 APRIL	SLIGHT
	URINE ABDOMEN	9 APRIL	SLIGHT
	PROSTRATE	9 APRIL	
	DEATH	9 APRIL	13.2 HOURS
85C00231	SQUINTING	9 APRIL	SLIGHT
	INACTIVE	9 APRIL	SLIGHT
	ROUGH COAT	9 APRIL	SLIGHT
85C00234	TREMORS	9 APRIL	MODERATE
	HUNCHED POSTURE	9 APRIL	SLIGHT
	SQUINTING	9 APRIL	MARKED
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	DEATH	9 APRIL	3.7 HOURS
85C00238	TWITCHING	9 APRIL	MODERATE
	HUNCHED POSTURE	9 APRIL	SLIGHT
	DEP. GRASPING REFLEX	9 APRIL	MODERATE
	SQUINTING	9 APRIL	SLIGHT
	DEATH	10 APRIL	24.3 HOURS

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE 2150 MG/KG TRIETHYLENEGLYCOL DINITRATE (CONT.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00242	TREMORS	9 APRIL	MARKED
	JUMPING	9 APRIL	MODERATE
	INACTIVE	9 APRIL	MARKED
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	DEP. RIGHTING REFLEX	9 APRIL	MODERATE
	PROSTRATE	10 APRIL	
	DEATH	10 APRIL	36.1 HOURS
85C00250	TREMORS	9 APRIL	MARKED
	INACTIVE	9 APRIL	MODERATE
	DEP. GRASPING REFLEX	9 APRIL	MODERATE
	SQUINTING	9 APRIL	MODERATE
	DEP. RIGHTING REFLEX	9 APRIL	SLIGHT
	DEATH	10 APRIL	24.5 HOURS
85C00251	INACTIVE	9 APRIL	MARKED
	HUNCHED POSTURE	9 APRIL	SLIGHT
	TREMORS	9 APRIL	SLIGHT
	DEP. GRASPING REFLEX	9 APRIL	SLIGHT
	SQUINTING	9 APRIL	MODERATE
	DEATH	9 APRIL	13.1 HOURS
85C00253	HUNCHED POSTURE	9 APRIL	SLIGHT
	DEP. GRASPING REFLEX	9 APRIL	SLIGHT
	ROUGH COAT	9 APRIL	SLIGHT
	INACTIVE	9 APRIL	SLIGHT

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE 2450 MG/KG TRIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00208	DEP. GRASPING REFLEX	9 APRIL	MODERATE
	INACTIVE	9 APRIL	MODERATE
	HUNCHED POSTURE	9 APRIL	SLIGHT
	SQUINTING	9 APRIL	SLIGHT
	URINE ABDOMEN	10 APRIL	SLIGHT
85C00209	HUNCHED POSTURE	9 APRIL	MODERATE
	INACTIVE	9 APRIL	MODERATE
	DEP. GRASPING REFLEX	9 APRIL	MODERATE
	SQUINTING	9 APRIL	MARKED
	TREMORS	9 APRIL	MARKED
	PROSTRATE	9 APRIL	
	DEATH	9 APRIL	4.3 HOURS
85C00212	INACTIVE	9 APRIL	MARKED
	SQUINTING	9 APRIL	SLIGHT
	HUNCHED POSTURE	9 APRIL	MODERATE
	TREMORS	9 APRIL	MODERATE
	DEATH	10 APRIL	24.1 HOURS
85C00215	INACTIVE	9 APRIL	SLIGHT
	URINE ABDOMEN	9 APRIL	MODERATE
	DEP. GRASPING REFLEX	9 APRIL	SLIGHT
	ROUGH COAT	9 APRIL	MODERATE
	SQUINTING	9 APRIL	SLIGHT
85C00213	HUNCHED POSTURE	9 APRIL	MODERATE
	TREMORS	9 APRIL	MARKED
	INACTIVE	9 APRIL	MARKED
	DEP. GRASPING REFLEX	9 APRIL	MODERATE
	SQUINTING	9 APRIL	MODERATE
	DEATH	9 APRIL	12.6 HOURS
85C00232	INACTIVE	9 APRIL	SLIGHT
	ROUGH COAT	9,16-23 APRIL	SLIGHT
	IRRITABLE	10 APRIL	MODERATE

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE 2450 MG/KG TRIETHYLENEGLYCOL DINITRATE (CONT.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00222	HUNCHED POSTURE	9 APRIL	MODERATE
	INACTIVE	9 APRIL	MODERATE
	TWITCHING	9 APIRL	MODERATE
	DEP. GRASPING REFLEX	9 APRIL	SLIGHT
	URINE ABDOMEN	9 APRIL	MODERATE
85C00241	TWITCHING	9 APRIL	SLIGHT
	INACTIVE	9 APRIL	MODERATE
	HUNCHED POSTURE	9 APRIL	MODERATE
	SQUINTING	9 APRIL	MODERATE
	DEP. GRASPING REFLEX	9 APRIL	SLIGHT
	DEATH	10 APRIL	24.4 HOURS
85C00257	INACTIVE	9 APRIL	MODERATE
	TREMORS	9 APRIL	MARKED
	HUNCHED POSTURE	9 APRIL	SLIGHT
	CLONIC CONVULSIONS	9 APRIL	MODERATE
	TONIC CONVULSIONS	9 APRIL	MODERATE
	DEP. GRASPING REFLEX	9 APRIL	MODERATE
	DEATH	9 APRIL	3.3 HOURS
85C00262	INACTIVE	9 APRIL	MARKED
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	DEP. RIGHTING REFLEX	9 APRIL	MARKED
	SQUINTING	9 APRIL	MODERATE
	DEATH	9 APRIL	3.3 HOURS

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE 2780 MG/KG TRIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00205	PROSTRATE	9 APRIL	
	TREMORS	9 APRIL	MARKED
	TONIC CONVULSION	9 APRIL	MARKED
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	SQUINTING	9 APRIL	MARKED
	DEATH	9 APRIL	3.0 HOURS
85C00207	TREMORS	9 APRIL	MODERATE
	HUNCHED POSTURE	9 APRIL	SLIGHT
	INACTIVE	9 APRIL	MODERATE
	DEP. RIGHTING REFLEX	9 APRIL	SLIGHT
	SQUINTING	9 APRIL	MARKED
	DEATH	9 APRIL	4.0 HOURS
85C00219	INACTIVE	9 APRIL	MODERATE
	HUNCHED POSTURE	9 APRIL	MODERATE
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	SQUINTING	9 APRIL	MARKED
	URINE ABDOMEN	9 APRIL	MODERATE
	TREMORS	9 APRIL	MARKED
	DEATH	9 APRIL	12.4 HOURS
85C00221	INACTIVE	9 APRIL	MARKED
	HUNCHED POSTURE	9 APRIL	SLIGHT
	TREMORS	9 APRIL	MARKED
	DEP. GRASPING REFLEX	9 APRIL	SLIGHT
	SQUINTING	9 APRIL	MODERATE
	DEATH	9 APRIL	12.4 HOURS
85C00224	INACTIVE	9 APRIL	MODERATE
	HUNCHED POSTURE	9 APRIL	SLIGHT
	TREMORS	9 APRIL	MODERATE
	DEP. GRASPING REFLEX	9 APRIL	MODERATE
	SQUINTING	9 APRIL	MODERATE
	URINE ABDOMEN	9 APRIL	SLIGHT
	PROSTRATE	9 APRIL	
	DEATH	9 APRIL	12.4 HOURS

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE 2780 MG/KG TRIETHYLENEGLYCOL DINITRATE (CONT.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00225	INACTIVE	9 APRIL	MARKED
	HUNCHED POSTURE	9 APRIL	MODERATE
	TREMORS	9 APRIL	MARKED
	SQUINTING	9 APRIL	MODERATE
	URINE ABDOMEN	9 APRIL	SLIGHT
	INC. STARTLE REFLEX	9 APRIL	MODERATE
	DEATH	9 APRIL	12.4 HOURS
85C00229	SQUINTING	9 APRIL	MARKED
	URINE ABDOMEN	9 APRIL	MODERATE
	TWITCHING	9 APRIL	MODERATE
	PROSTRATE	9 APRIL	
	DEATH	9 APRIL	2.9 HOURS
85C00237	TREMORS	9 APRIL	MARKED
	JUMPING	9 APRIL	MARKED
	HUNCHED POSTURE	9 APRIL	SLIGHT
	INC. STARTLE REFLEX	9 APRIL	MARKED
	DEP. GRASPING REFLEX	9 APRIL	SLIGHT
	SQUINTING	9 APRIL	MODERATE
	DEATH	9 APRIL	3.4 HOURS
85C00260	INACTIVE	9 APRIL	MARKED
	TREMORS	9 APRIL	MARKED
	JUMPING	9 APRIL	MODERATE
	TONIC CONVULSION	9 APRIL	MARKED
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	DEP. RIGHTING REFLEX	9 APRIL	MARKED
	SQUINTING	9 APRIL	MARKED
	URINE ABDOMEN	9 APRIL	MODERATE
	DEATH	9 APRIL	2.9 HOURS
85C00261	INACTIVE	9 APRIL	MARKED
	TREMORS	9 APRIL	MARKED
	HUNCHED POSTURE	9 APRIL	SLIGHT
	ROUGH COAT	9 APRIL	MODERATE
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	DEP. RIGHTING REFLEX	9 APRIL	SLIGHT
	SQUINTING	9 APRIL	MARKED
	URINE ABDOMEN	9 APRIL	SLIGHT
	PROSTRATE	9 APRIL	
	DEATH	9 APRIL	5.6 HOURS

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE 3160 MG/KG TRIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00206	INACTIVE	9 APRIL	MARKED
	HUNCHED POSTURE	9 APRIL	MODERATE
	TREMORS	9 APRIL	MODERATE
	INC. STARTLE REFLEX	9 APRIL	MODERATE
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	DEP. RIGHTING REFLEX	9 APRIL	MODERATE
	SQUINTING	9 APRIL	MARKED
	DEATH	9 APRIL	2.6 HOURS
85C00210	TREMORS	9 APRIL	MODERATE
	HUNCHED POSTURE	9 APRIL	SLIGHT
	INACTIVE	9 APRIL	MODERATE
	DEP. GRASPING REFLEX	9 APRIL	MODERATE
	SQUINTING	9 APRIL	MODERATE
	INC. STARTLE REFLEX	9 APRIL	MARKED
	DEATH	10 APRIL	31.5 HOURS
85C00214	PROSTRATE	9 APRIL	
	INC. STARTLE REFLEX	9 APRIL	MODERATE
	SQUINTING	9 APRIL	MARKED
	DEATH	9 APRIL	12.0 HOURS
85C00233	PROSTRATE	9 APRIL	
	TREMORS	9 APRIL	MARKED
	SQUINTING	9 APRIL	MODERATE
	DEATH	9 APRIL	2.6 HOURS
85C00248	INACTIVE	9 APRIL	MODERATE
	HUNCHED POSTURE	9 APRIL	SLIGHT
	TREMORS	9 APRIL	MODERATE
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	SQUINTING	9 APRIL	MARKED
	URINE ABDOMEN	9 APRIL	SLIGHT
	PROSTRATE	9 APRIL	
	DEATH	9 APRIL	5.3 HOURS
85C00252	DEATH	9 APRIL	1.4 HOURS

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE 3160 MG/KG TRIETHYLENEGLYCOL DINITRATE (CONT.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00254	INACTIVE	9 APRIL	MARKED
	HUNCHED POSTURE	9 APRIL	SLIGHT
	TWITCHING	9 APRIL	SLIGHT
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	DEP. RIGHTING REFLEX	9 APRIL	MODERATE
	SQUINTING	9 APRIL	MARKED
	URINE ABDOMEN	9 APRIL	SLIGHT
	DEATH	9 APRIL	5.2 HOURS
85C00216	PROSTRATE	9 APRIL	
	TREMORS	9 APRIL	MARKED
	INCREASED SALIVATION	9 APRIL	MODERATE
	URINE ABDOMEN	9 APRIL	MODERATE
	SQUINTING	9 APRIL	MODERATE
	DEATH	9 APRIL	2.5 HOURS
85C00220	INACTIVE	9 APRIL	MODERATE
	HUNCHED POSTURE	9 APRIL	SLIGHT
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	SQUINTING	9 APRIL	MODERATE
	TREMORS	9 APRIL	MODERATE
	DEATH	9 APRIL	5.5 HOURS
85C00230	INACTIVE	9 APRIL	MODERATE
	HUNCHED POSTURE	9 APRIL	MODERATE
	TREMORS	9 APRIL	MODERATE
	DEP. GRASPING REFLEX	9 APRIL	MODERATE
	SQUINTING	9 APRIL	MODERATE
	DEATH	9 APRIL	5.6 HOURS

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE CONTROL TRIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00268†	NORMAL	8-23 APRIL	
85C00300†	NORMAL	8-23 APRIL	
85C00307†	NORMAL	8-23 APRIL	
85C00158	NORMAL	4-19 FEBRUARY	
85C00166	NORMAL	4-19 FEBRUARY	
85C00169	MISDOSE		
85C00173	IRRITABLE	6 FEBRUARY	SLIGHT
	TREMORS	7 FEBRUARY	SLIGHT
85C00184	NORMAL	4-19 FEBRUARY	

† Cage controls, remaining animals are vehicle controls.

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE 1290 MG/KG TRIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00267	NORMAL	8-26 APRIL	
85C00274	TREMORS	12 APRIL	SLIGHT
	SQUINTING	12 APRIL	MARKED
	INACTIVE	12 APRIL	MODERATE
	HUNCHED POSTURE	12 APRIL	MODERATE
	DEP. GRASPING REFLEX	12 APRIL	MARKED
85C00288	NORMAL	8-26 APRIL	
85C00297	HUNCHED POSTURE	12 APRIL	MARKED
	INACTIVE	12 APRIL	MARKED
	TREMORS	12 APRIL	MODERATE
	SQUINTING	12 APRIL	MARKED
	DEP. GRASPING REFLEX	12 APRIL	MARKED
85C00323	INACTIVE	12 APRIL	SLIGHT
	HUNCHED POSTURE	12 APRIL	SLIGHT
	SQUINTING	12 APRIL	SLIGHT

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE 1670 MG/KG TRIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00265	NORMAL	8-24 APRIL	
85C00266	INACTIVE	10-11 APRIL	MARKED
	INCEASED STARTLE REFLEX	10 APRIL	MODERATE
	DEP. GRASPING REFLEX	10-11 APRIL	MODERATE
	SQUINTING	10-11 APRIL	MARKED
	TREMORS	10 APRIL	MODERATE
	URINE ABDOMEN	10-11 APRIL	MODERATE
	DEATH	11 APRIL	29.2 HRS
85C00269	NORMAL	8-24 APRIL	
85C00272	NORMAL	8-24 APRIL	
85C00280	NORMAL	8-24 APRIL	
85C00292	NORMAL	8-24 APRIL	
85C00293	INACTIVE	10 APRIL	MARKED
	HUNCHED POSTURE	10 APRIL	MODERATE
	TREMORS	10 APRIL	MODERATE
	SQUINTING	10 APRIL	MARKED
	DEP. GRASPING REFLEX	10 APRIL	MODERATE
	DEATH	11 APRIL	22.0 HOURS
85C00308	INACTIVE	10-11APRIL	MARKED
	HUNCHED POSTURE	10-11APRIL	SLIGHT
	TREMORS	10 APRIL	MARKED
	SQUINTING	10-11APRIL	MARKED
	DEP. GRASPING REFLEX	10-11APRIL	MARKED
	DEATH	11 APRIL	45.3 HOURS
85C00317	URINE ABDOMEN	10 APRIL	SLIGHT
85C00321	NORMAL	10-24 APRIL	

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE 2150 MG/KG TRIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00270	TREMORS	9 APRIL	MARKED
	INACTIVE	9 APRIL	MARKED
	INC. STARTLE REFLEX	9 APRIL	MARKED
	DEP. GRASPING REFLEX	9 APRIL	SLIGHT
	SQUINTING	9 APRIL	SLIGHT
	DEATH	9 APRIL	3.7 HOURS
85C00277	HUNCHED POSTURE	9 APRIL	SLIGHT
	SQUINTING	9 APRIL	SLIGHT
	INACTIVE	9 APRIL	MODERATE
	IRRITABLE	17 APRIL	SLIGHT
85C00279	PROSTRATE	9 APRIL	
	TREMORS	9 APRIL	MARKED
	SQUINTING	9 APRIL	MODERATE
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	DEATH	9 APRIL	3.6 HOURS
85C00283	ROUGH COAT	9 APRIL	SLIGHT
85C00295	DEATH	9 APRIL	1.8 HOURS
85C00296	HUNCHED POSTURE	9 APRIL	SLIGHT
	JUMPING	9 APRIL	SLIGHT
	INC. STARTLE REFLEX	9 APRIL	MARKED
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	DEP. RIGHTING REFLEX	9 APRIL	MODERATE
	SQUINTING	9 APRIL	MODERATE
	DEATH	9 APRIL	3.7 HOURS
85C00299	INACTIVE	9 APRIL	MODERATE
	HUNCHED POSTURE	9 APRIL	SLIGHT
	TREMORS	9 APRIL	MARKED
	DEP. RIGHTING REFLEX	9 APRIL	MODERATE
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	INC. STARTLE REFLEX	9 APRIL	MARKED
	SQUINTING	9 APRIL	MARKED
	DEATH	9 APRIL	4.3 HOURS

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE 2150 MG/KG TRIETHYLENEGLYCOL DINITRATE (CONT.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00303	HUNCHED POSTURE	9 APRIL	MODERATE
	INACTIVE	9 APRIL	MODERATE
	TWITCHING	9 APRIL	SLIGHT
	DEP. GRASPING REFLEX	9 APRIL	SLIGHT
	SQUINTING	9 APRIL	MODERATE
	TREMORS	9 APRIL	MARKED
	PROSTRATE	9 APRIL	
	DEATH	9 APRIL	13.1 HOURS
85C00318	INACTIVE	9 APRIL	MODERATE
	HUNCHED POSTURE	9 APRIL	MODERATE
	TREMORS	9 APRIL	MODERATE
	DEP. GRASPING REFLEX	9 APRIL	SLIGHT
	URINE ABDOMEN	9 APRIL	SLIGHT
	DEATH	9 APRIL	13.1 HOURS
85C00322	HUNCHED POSTURE	9 APRIL	MODERATE
	INACTIVE	9 APRIL	MARKED
	DEP. GRASPING REFLEX	9 APRIL	SLIGHT
	SQUINTING	9 APRIL	SLIGHT
	IRRITABLE	10 APRIL	SLIGHT

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE 2450 MG/KG TRIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00276	INACTIVE	9 APRIL	MODERATE
	HUNCHED POSTURE	9 APRIL	SLIGHT
	TREMORS	9 APRIL	MARKED
	JUMPING	9 APRIL	SLIGHT
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	DEP. RIGHTING REFLEX	9 APRIL	MODERATE
	INC. STARTLE REFLEX	9 APRIL	SLIGHT
	SQUINTING	9 APRIL	SLIGHT
85C00281	DEATH	9 APRIL	3.3 HOURS
85C00282	INACTIVE	9 APRIL	MARKED
	HUNCHED POSTURE	9 APRIL	SLIGHT
	TREMORS	9 APRIL	MODERATE
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	DEATH	9 APRIL	5.9 HOURS
85C00285	DEATH	9 APRIL	3.3 HOURS
85C00290	HUNCHED POSTURE	9 APRIL	MODERATE
	TWITCHING	9 APRIL	MARKED
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	SQUINTING	9 APRIL	MODERATE
	INACTIVE	9 APRIL	MARKED
	DEATH	10 APRIL	22.0 HOURS
85C00301	TREMORS	9 APRIL	MARKED
	INACTIVE	9 APRIL	MARKED
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	SQUINTING	9 APRIL	MODERATE
	URINE ABDOMEN	9 APRIL	MODERATE
	PROSTRATE	9 APRIL	
	DEATH	9 APRIL	12.8 HOURS
85C00306	HUNCHED POSTURE	9 APRIL	SLIGHT
	TREMORS	9 APRIL	MODERATE
	INACTIVE	9 APRIL	SLIGHT
	TREMORS	9 APRIL	MARKED
	PROSTRATE	9 APRIL	
	DEATH	9 APRIL	6.9 HOURS

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE 2450 MG/KG TRIETHYLENEGLYCOL DINITRATE (CONT.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00312	INACTIVE	9 APRIL	MODERATE
	TREMORS	9 APRIL	MARKED
	HUNCHED POSTURE	9 APRIL	SLIGHT
	DEP. GRASPING REFLEX	9 APRIL	MODERATE
	SQUINTING	9 APRIL	MODERATE
	DEATH	9 APRIL	3.3 HOURS
85C00316	PROSTRATE	9 APRIL	
	TREMORS	9 APRIL	MARKED
	TONIC CONVULSION	9 APRIL	MARKED
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	SQUINTING	9 APRIL	MODERATE
	DEATH	9 APRIL	2.4 HOURS
85C00320	DEATH	9 APRIL	1.6 HOURS

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE 2780 MG/KG TRIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00289	MORIBUND	9 APRIL	
	DEATH	9 APRIL	2.9 HOURS
85C00291	INACTIVE	9 APRIL	MARKED
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	DEP. RIGHTING REFLEX	9 APRIL	MARKED
	SQUINTING	9 APRIL	MARKED
	URINE ABDOMEN	9 APRIL	MARKED
	DEATH	9 APRIL	2.9 HOURS
85C00304	INACTIVE	9 APRIL	MODERATE
	HUNCHED POSTURE	9 APRIL	SLIGHT
	TREMORS	9 APRIL	MODERATE
	DEATH	9 APRIL	12.4 HOURS
85C00305	DEATH	9 APRIL	2.0 HOURS
85C00271	INACTIVE	9 APRIL	MODERATE
	HUNCHED POSTURE	9 APRIL	SLIGHT
	TREMORS	9 APRIL	MARKED
	ALOPECIA, HEAD	9 APRIL	SLIGHT
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	SQUINTING	9 APRIL	MODERATE
	TREMORS	9 APRIL	MODERATE
	PROSTRATE	9 APRIL	
	DEATH	9 APRIL	12.5 HOURS
85C00275	TREMORS	9 APRIL	MARKED
	PROSTRATE	9 APRIL	
	DEATH	9 APRIL	5.5 HOURS
85C00286	INACTIVE	9 APRIL	MARKED
	TREMORS	9 APRIL	MARKED
	DEP. RIGHTING REFLEX	9 APRIL	MODERATE
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	SQUINTING	9 APRIL	MODERATE
	URINE ABDOMEN	9 APRIL	SLIGHT
	DEATH	9 APRIL	3.5 HOURS

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE 2780 MG/KG TRIETHYLENEGLYCOL DINITRATE (CONT.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00311	INACTIVE	9 APRIL	MARKED
	TREMORS	9 APRIL	MARKED
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	DEP. RIGHTING REFLEX	9 APRIL	MARKED
	SQUINTING	9 APRIL	MARKED
	DEATH	9 APRIL	3.6 HOURS
85C00314	TREMORS	9 APRIL	MARKED
	PROSTRATE	9 APRIL	
	DEATH	9 APRIL	2.9 HOURS
85C00315	DEATH	9 APRIL	2.0 HOURS

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE 3160 MG/KG TRIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85C00287	DEATH	9 APRIL	1.5 HOURS
85C00294	DEATH	10 APRIL	23.7 HOURS
85C00298	DEATH	9 APRIL	1.6 HOURS
85C00309	DEATH	9 APRIL	1.6 HOURS
85C00313	DEATH	9 APRIL	1.7 HOURS
85C00319	MORIBUND	9 APRIL	
	DEATH	9 APRIL	2.6 HOURS
85C00324	INACTIVE	9 APRIL	MARKED
	TREMORS	9 APRIL	MARKED
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	DEP. RIGHTING REFLEX	9 APRIL	MODERATE
	SQUINTING	9 APRIL	MARKED
	DEATH	9 APRIL	2.6 HOURS
85C00273	DEATH	9 APRIL	1.5 HOURS
85C00278	MORIBUND	9 APRIL	
	DEATH	9 APRIL	2.6 HOURS
85C00284	INACTIVE	9 APRIL	MARKED
	HUNCHED POSTURE	9 APRIL	SLIGHT
	TREMORS	9 APRIL	MARKED
	DEP. GRASPING REFLEX	9 APRIL	MARKED
	SQUINTING	9 APRIL	SLIGHT
	PROSTRATE	9 APRIL	
	DEATH	9 APRIL	5.2 HOURS

Appendix F: INDIVIDUAL BODY WEIGHTS IN GRAMS

Males: 1290 mg/kg

Animal Number	Receipt	Dosing	Termination Day 4	Termination Day 14
85C00236	28	32	33	35
85C00240	27	32	33	34
85C00246	26	34	36	35
85C00247	26	31	32	35
85C00249	28	33	34	36
Mean	27.0	32.4	33.6	35.0
Standard Deviation	1.0	1.1	1.5	0.7
Standard Error of the Means	0.4	0.5	0.7	0.3

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Males: 1670 mg/kg

Animal Number	Receipt	Dosing	Termination Day 7	Termination Day 14
85C00218	28	27	29	31
85C00227	29	32	34	36
85C00228	25	29	31	34
85C00235	30	31	Dead	
85C00239	27	31	34	35
85C00245	28	29	Dead	
85C00255	25	27	30	34
85C00256	29	34	37	37
85C00263	25	27	29	30
85C00264	24	29	31	21
Mean	27.0	29.6	31.9	32.3
Standard Deviation	2.1	2.4	2.9	5.1
Standard Error of the Means	0.7	0.8	1.0	1.8

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Males: 2150 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14
85C00217	25	28	Dead	
85C00223	31	33	35	36
85C00226	26	28	Dead	
85C00231	27	33	34	34
85C00234	26	32	Dead	
85C00238	26	30	Dead	
85C00242	27	30	Dead	
85C00250	26	29	Dead	
85C00251	31	34	Dead	
85C00253	33	36	43	41
Mean	27.8	31.3	37.3	37.0
Standard Deviation	2.8	2.7	4.9	3.6
Standard Error of the Means	0.9	0.9	2.9	2.1

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Males: 2450 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14
85C00208	27	28	30	32
85C00209	30	27	Dead	
85C00212	25	26	Dead	
85C00213	25	27	Dead	
85C00215	29	34	35	38
85C00222	28	30	31	33
85C00232	28	32	37	37
85C00241	29	33	Dead	
85C00257	28	32	Dead	
85C00262	27	31	Dead	
Mean	27.6	30.0	33.3	35.0
Standard Deviation	1.7	2.8	3.3	2.9
Standard Error of the Means	0.5	0.9	1.6	1.5

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Males: 2780 mg/kg

Animal Number	Receipt	Dosing	Termination Day 7	Termination Day 14
85C00205	27	28	Dead	
85C00207	28	30	Dead	
85C00219	26	28	Dead	
85C00221	32	34	Dead	
85C00224	29	33	Dead	
85C00225	27	31	Dead	
85C00229	27	31	Dead	
85C00237	31	34	Dead	
85C00260	29	30	Dead	
85C00261	26	29	Dead	
Mean	28.2	30.8		
Standard Deviation	2.0	2.3		
Standard Error of the Means	0.6	0.7		

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Males: 3160 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14
85C00206	26	27	Dead	
85C00210	26	27	Dead	
85C00214	26	27	Dead	
85C00216	26	26	Dead	
85C00220	30	31	Dead	
85C00230	26	26	Dead	
85C00233	28	31	Dead	
85C00248	28	30	Dead	
85C00252	29	31	Dead	
85C00254	26	30	Dead	
Mean	27.1	28.6		
Standard Deviation	1.5	2.2		
Standard Error of the Means	0.5	0.7		

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Females: 1290 mg/kg

Animal Number	Receipt	Dosing	Day 4	Termination Day 14
85C00267	26	28	32	31
85C00274	25	27	28	30
85C00288	26	27	28	29
85C00297	29	30	32	31
85C00323	24	27	29	30
Mean	26.0	27.8	29.8	30.2
Standard Deviation	1.9	1.3	2.1	0.8
Standard Error of the Means	0.8	0.6	0.9	0.4

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Females: 1670 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14
85C00265	26	26	28	30
85C00266	28	30	Dead	
85C00269	28	30	31	34
85C00272	25	25	28	29
85C00280	29	31	32	34
85C00292	25	26	26	27
85C00293	24	25	Dead	
85C00308	28	28	Dead	
85C00317	25	26	29	29
85C00321	25	27	30	30
Mean	26.3	27.4	31.3	30.4
Standard Deviation	1.8	2.2	1.2	2.6
Standard Error of the Means	0.6	0.7	0.7	1.0

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Females: 2150 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14
85C00270	29	29	Dead	
85C00277	27	30	32	31
85C00279	25	27	Dead	
85C00283	27	30	32	33
85C00295	25	27	Dead	
85C00296	29	29	Dead	
85C00299	31	32	Dead	
85C00303	30	31	Dead	
85C00318	23	26	Dead	
85C00322	24	26	30	31
Mean	27.0	28.7	31.3	31.7
Standard Deviation	2.7	2.1	1.2	1.2
Standard Error of the Means	0.9	0.7	0.7	0.7

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Females: 2450 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14
85C00276	28	31	Dead	
85C00281	25	26	Dead	
85C00282	30	31	Dead	
85C00285	28	29	Dead	
85C00290	26	28	Dead	
85C00301	26	28	Dead	
85C00306	23	24	Dead	
85C00312	23	24	Dead	
85C00316	24	27	Dead	
85C00320	26	26	Dead	
Mean	25.9	27.4		
Standard Deviation	2.3	2.5		
Standard Error of the Means	0.7	0.8		

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Females: 2780 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14
85C00271	25	27	Dead	
85C00275	29	29	Dead	
85C00286	25	26	Dead	
85C00289	19	23	Dead	
85C00291	25	26	Dead	
85C00304	27	29	Dead	
85C00305	21	23	Dead	
85C00311	29	31	Dead	
85C00314	26	27	Dead	
85C00315	24	26	Dead	
Mean	25.0	26.7		
Standard Deviation	3.2	2.5		
Standard Error of the Means	1.0	0.8		

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Females: 3160 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14
85C00273	26	29	Dead	
85C00278	25	26	Dead	
85C00284	27	27	Dead	
85C00287	28	28	Dead	
85C00294	25	26	Dead	
85C00298	25	25	Dead	
85C00309	29	31	Dead	
85C00313	27	24	Dead	
85C00319	26	28	Dead	
85C00324	23	24	Dead	
Mean	25.0	26.8		
Standard Deviation	3.2	2.3		
Standard Error of the Means	1.0	0.7		

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Vehicle Control

Animal Number	Receipt	Dosing	Termination Day 8	Termination Day 14
Males				
85C00074	25	33	36	36
85C00097	26	31	34	35
85C00103	25	32	34	34
85C00114	24	32	35	36
85C00133	26	32	34	34
Mean	25.2	32.0	34.6	35.0
Standard Deviation	0.8	0.7	0.9	1.0
Standard Error of the Mean	0.4	0.3	0.4	0.5
Females				
85C00158	21	26	27	27
85C00166	24	24	26	29
85C00169	21	24	Misdosed	
85C00173	22	26	27	29
85C00184	24	25	26	29
Mean	22.4	25.0	26.5	28.5
Standard Deviation	1.5	1.0	0.6	1.0
Standard Error of the Mean	0.7	0.5	0.3	0.5

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Cage Control

Animal Number	Receipt	Dosing*	Termination Day 8	Termination Day 14
Males				
85C00243	27	34	35	37
85C00244	26	32	32	32
85C00258	27	32	34	34
85C00259	30	35	36	35
Mean	27.5	33.3	34.3	34.5
Standard Deviation	1.7	1.5	1.7	2.1
Standard Error of the Mean	0.9	0.8	0.9	1.1
Females				
85C00268	26	30	30	29
85C00300	29	31	30	31
85C00307	25	29	31	29
Mean	26.7	30.0	30.3	29.7
Standard Deviation	2.1	1.0	0.6	1.6
Standard Error of the Mean	1.1	0.6	0.3	0.7

*Weights are from the end of quarantine, the day before dosing.

Appendix G: PATHOLOGY REPORT

GLP Study 84010
Oral Lethal Dose Test in Mice of Triethyleneglycol Dinitrate

History: This study was designed to determine the oral toxicity of triethyleneglycol dinitrate (TEGDN), in male and female ICR mice weighing approximately 30 grams. After accumulation and randomization, these animals were dosed by oral gavage in corn oil vehicle as follows:

Vehicle Control - Vehicle only
Group 1 - 2150 mg/kg
Group 2 - 2450 mg/kg
Group 3 - 2780 mg/kg
Group 4 - 3160 mg/kg
Group 5 - 1670 mg/kg
Group 6 - 1290 mg/kg
Group 7 - Cage Controls

Gross Necropsy Findings:

VEHICLE CONTROL
MALES

LAIR ACCESSION #	ID #	GROSS FINDINGS
36844	85C00074	Live - Not remarkable (NR)
36852	85C00097	Live - NR
36854	85C00103	Live - NR
36857	85C00114	Live - NR
36867	85C00133	Live - NR

DOSE GROUP 1 - 2150 mg/kg
MALES

37200	85C00217	Dead - NR
37451	85C00223	Live - NR
37246	85C00226	Dead - NR
37452	85C00231	Live - NR
37203	85C00234	Dead - NR
37325	85C00238	Dead - NR
37328	85C00242	Dead - NR
37322	85C00250	Dead - NR
37250	85C00251	Dead - NR
37456	85C00253	Live - NR

Appendix G: PATHOLOGY REPORT (cont.)

DOSE GROUP 2 - 2450 mg/kg
MALES

LAIR ACCESSION #	ID #	GROSS FINDINGS
37448	85C00208	Live - NR
37236	85C00209	Dead - NR
37324	85C00212	Dead - NR
37239	85C00213	Dead - NR
37449	85C00215	Live - NR
37450	85C00222	Live - NR
37453	85C00232	Live - NR
37327	85C00241	Dead - NR
37205	85C00257	Dead - NR
37207	85C00262	Dead - NR

DOSE GROUP 3 - 2780 mg/kg
MALES

37197	85C00205	Dead - NR
37237	85C00207	Dead - NR
37241	85C00219	Dead - NR
37243	85C00221	Dead - NR
37244	85C00224	Dead - NR
37245	85C00225	Dead - NR
37201	85C00229	Dead - NR
37248	85C00237	Dead - NR
37206	85C00260	Dead - NR
37252	85C00261	Dead - NR

DOSE GROUP 4 - 3160 mg/kg
MALES

37198	85C00206	Dead - NR
37238	85C00210	Dead - NR
37240	85C00214	Dead - NR
37199	85C00216	Dead - NR
37242	85C00220	Dead - NR
37247	85C00230	Dead - NR
37202	85C00233	Dead - NR
37249	85C00248	Dead - NR
37204	85C00252	Dead - NR
37251	85C00254	Dead - NR

Appendix G: PATHOLOGY REPORT (cont.)

DOSE GROUP 5 - 1670 mg/kg
MALES

LAIR ACCESSION #	ID #	GROSS FINDINGS
37467	85C00218	Live - NR
37468	85C00227	Live - NR
37469	85C00228	Live - NR
37326	85C00235	Dead - NR
37470	85C00239	Live - NR
37332	85C00245	Dead - NR
37471	85C00255	Live - NR
37472	85C00256	Live - NR
37473	85C00263	Live - NR
37474	85C00264	Live - NR

DOSE GROUP 6 - 1290 mg/kg
MALES

37484	85C00236	Live - NR
37485	85C00240	Live - NR
37486	85C00246	Live - NR
37487	85C00247	Live - NR
37488	85C00249	Live - NR

DOSE GROUP 7 - CAGE CONTROL
MALES

37454	85C00243	Live - NR
37455	85C00244	Live - NR
37457	85C00258	Live - NR
37458	85C00259	Live - NR

VEHICLE CONTROL
FEMALES

36877	85C00158	Live - NR
36878	85C00166	Live - NR
36880	85C00173	Live - NR
36887	85C00184	Live - NR

Appendix G: PATHOLOGY REPORT (cont.)

DOSE GROUP 1 - 2150 mg/kg
FEMALES

LAIR ACCESSION #	ID #	GROSS FINDINGS
37208	85C00270	Dead - NR
37460	85C00277	Live - NR
37212	85C00279	Dead - NR
37461	85C00283	Live - NR
37218	85C00295	Dead - NR
37219	85C00296	Dead - NR
37234	85C00299	Dead - NR
37259	85C00303	Dead - NR
37262	85C00318	Dead - NR
37464	85C00322	Live - NR

DOSE GROUP 2 - 2450 mg/kg
FEMALES

37210	85C00276	Dead - NR
37213	85C00281	Dead - NR
37255	85C00282	Dead - NR
37214	85C00285	Dead - NR
37257	85C00290	Dead - NR
37258	85C00301	Dead - NR
37261	85C00306	Dead - NR
37223	85C00312	Dead - NR
37227	85C00316	Dead - NR
37229	85C00320	Dead - NR

DOSE GROUP 3 - 2780 mg/kg
FEMALES

37253	85C00271	Dead - NR
37254	85C00275	Dead - NR
37233	85C00286	Dead - NR
37216	85C00289	Dead - NR
37217	85C00291	Dead - NR
37260	85C00304	Dead - NR
37221	85C00305	Dead - NR
37235	85C00311	Dead - NR
37225	85C00314	Dead - NR
37226	85C00315	Dead - NR

Appendix G: PATHOLOGY REPORT (cont.)

DOSE GROUP 4 - 3160 mg/kg
FEMALES

LAIR ACCESSION #	ID #	GROSS FINDINGS
37209	85C00273	Dead - NR
37211	85C00278	Dead - NR
37256	85C00284	Dead - NR
37215	85C00287	Dead - NR
37323	85C00294	Dead - NR
37220	85C00298	Dead - NR
37222	85C00309	Dead - NR
37224	85C00313	Dead - NR
37228	85C00319	Dead - NR
37230	85C00324	Dead - NR

DOSE GROUP 5 - 1670 mg/kg
FEMALES

37475	85C00265	Live - NR
37333	85C00266	Dead - NR
37476	85C00269	Live - NR
37477	85C00272	Live - NR
37478	85C00280	Live - NR
37479	85C00292	Live - NR
37329	85C00293	Dead - NR
37334	85C00308	Dead - NR
37480	85C00317	Live - NR
37481	85C00321	Live - NR

DOSE GROUP 6 - 1290 mg/kg
FEMALES

37489	85C00267	Live - NR
37491	85C00274	Live - NR
37490	85C00288	Live - NR
37492	85C00297	Live - NR
37493	85C00323	Live - NR

DOSE GROUP 7 - CAGE CONTROL
FEMALES

37459	85C00268	Live - NR
37462	85C00300	Live - NR
37463	85C00307	Live - NR

Appendix G: PATHOLOGY REPORT (cont.)

Summary: Thirty-five of the 55 males exposed to TEGDN were received dead for necropsy within 48 hours after dosing. Five males exposed to vehicle and 4 males serving as cage controls were received live at the end of the study, along with survivors from the dosed groups.

Forty of the 55 females exposed to TEGDN were received dead for necropsy within 48 hours after dosing. Four vehicle control and three cage controls were received live at the end of the study, along with survivors from the dosed groups.

No remarkable gross lesions were recognized in any of the animals in this study.

Microscopic Findings: No tissue was taken for microscopic evaluation.

Conclusions: The acute administration of TEGDN did not produce any remarkable gross lesions in male or female mice dying on study or terminated at the conclusion of the study.

Robert L. Morrissey

ROBERT L. MORRISSEY, DVM
LTC, VC
USAR

Lance O. Lollini

LANCE O. LOLLINI, DVM
LTC, VC
Chief, Pathology Services Group

30 July 1985

Distribution List

Commander
US Army Biomedical Research and Development Laboratory (27)
ATTN: SGRD-UBZ-C
Fort Detrick, Frederick, MD 21701-5010

Defense Technical Information Center (DTIC) (2)
ATTN: DTIC-DLA
Cameron Station
Alexandria, VA 22304-6145

US Army Medical Research and Development Command (2)
ATTN: SGRD-RMI-S
Fort Detrick, Frederick, MD 21701-5012

Commandant
Academy of Health Sciences, US Army
ATTN: AHS-CDM
Fort Sam Houston, TX 78234

Chief
USAEHA Regional Division, West
Fitzsimmons AMC
Aurora, CO 80045

Chief
USAEHA Regional Division, North
Fort George G. Meade, MD 20755

Chief
USAEHA Regional Division, South
Bldg. 180
Fort McPherson, GA 30330

Commander
USA Health Services Command
ATTN: HSPA-P
Fort Sam Houston, TX 78234

Commander US Army Materiel Command
ATTN: AMSCG
5001 Eisenhower Avenue
Alexandria, VA 22333

Commander
US Army Environmental Hygiene Agency
ATTN: Librarian, HSDH-AD-L
Aberdeen Proving Ground, MD 21010

Dean
School of Medicine
Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20014

Commander
US Army Materiel Command
ATTN: AMCEN-A
5001 Eisenhower Avenue
Alexandria, VA 22333

HQDA
ATTN: DASG-PSP-E
Falls Church, VA 22041-3258

HQDA
ATTN: DAEN-RDM
20 Massachusetts, NW
Washington, D.C. 20314

CDR, US Army Toxic and Hazardous Material Agency
ATTN: DRXTH/ES
Aberdeen Proving Ground, MD 21010

Commandant
Academy of Health Sciences
United States Army
ATTN: Chief, Environmental Quality Branch
Preventive Medicine Division (HSHA-IPM)
Fort Sam Houston, TX 78234